Table 1. *Ranunculus* species used for research.

Ingroup
Ranunculus subg. *Ranunculus* Peterm. 1846.
Sect. *Pseudadonis* F. Muell. 1860.
Syn.: Sect. *Epirotes* (Prantl) L. Benson 1936. 26–33, 169–176.
Alpine group Fisher, Alp. Ran. New Zealand 1965.
R. anemoneus F. Muell., Trans. Philos. Soc. Victoria 1855. 1: 97.
R. buchananii Hook. f., Handbk. New Zealand Fl. 1864. 5.
R. crithmifolius Hook. f., Handbk. New Zealand Fl. 1864. 6.
subsp. *crithmifolius* Fisher, Alp. Ran. New Zealand 1965.
subsp. *paucifolius* (T. Kirk) Fisher, Alp. Ran. New Zealand 1965.
R. enysii T. Kirk, Trans. New Zealand Inst. 1880. 12: 394.
R. godleyanus Hook. f., Handbk. New Zealand Fl. 1867. 723.
R. gracilipes Hook. f., Handb. New Zealand Fl. 1864. 8.
R. grahamii Petrie, Trans. New Zealand Inst. 1914. 46: 32.
R. gunnianus Hook., Hook. J. Bot. 1834. 1: 244.
R. haastii Hook. f., Handbk. New Zealand Fl. 1864. 6.
subsp. *haastii* Fisher, Alp. Ran. New Zealand 1965.
subsp. *piliferus* Fisher, Alp. Ran. New Zealand 1965.
R. insignis Hook. f., Fl. New Zealand 1852. 1: 8.
R. lyallii Hook. f., Handbk. New Zealand Fl. 1864. 4.
R. nivicola Hook., Ic. 1844. 571–572.
R. pachyrrhizus Hook. f., Handbk. New Zealand Fl. 1864. 8.
R. pinguis Hook. f., Fl. Antarct. 1844. 3.
R. scrithalis P. J. Garnock-Jones, New Zealand J. Bot. 1987. 25: 126.
R. sericophyllus Hook. f., Handbk. New Zealand Fl. 1864. 6.
R. verticillatus T. Kirk, Fl. New Zealand 1899. 13.
R. viridis H. D. Wilson & P. J. Garnock-Jones, New Zealand J. Bot. 1983. 21: 342.
Outgroups
Ranunculus subg. *Ranunculus* Peterm. 1846.
Sect. *Pseudadonis* F. Muell. 1860.
Syn.: Sect. *Epirotes* (Prantl) Benson 1936. 26–33, 169–176.
Lowland group Fisher, Alp. Ran. New Zealand 1965.
R. acaulis Banks & Soland. ex DC., Re. Veg. Syst. Nat. 1817. 1: 270.
Sect. *Hecatonia* (Lour.) DC. 1824.
R. sceleratus L., Sp. Pl. 1753. 551.
Sect. *Chrysanthe* (Spach) L. Benson 1936.
R. recens T. Kirk, Fl. New Zealand 1899. 13.
Ranunculus subg. *Batrachium* (DC.) Peterm. 1846.
R. circinatus Sibth., Fl. Oxon. 1794. 175.

cies are found in habitats ranging from those above the permanent snow line, on stone or silt screes, to grassland, swamps, or sheltered situations. Breeding studies carried out on most, but not all species have suggested the existence of two reproductively incompatible groups of species (Fisher, 1965). Following the onset of mountain building in the Pliocene (Cooper & Millener, 1993; Batt et al., 2000), diversification in different breeding groups is thought to have led to the evolution of similar morphologies in species occupying similar alpine niches (Fisher, 1965). This is considered to have been a consequence of alpine geological boundaries, which limited seed dispersal and restricted species from occupying suitable but geographically disjunct sites in different parts of New Zealand (Fisher, 1965). Origins of the alpine Ranunculi of New Zealand are unclear, and their distribution between Australia, New Zealand, and their subantarctic islands has given rise to different hypotheses of evolutionary relationships and long-distance dispersal (Fisher, 1965; Raven, 1973; Wardle, 1978).

Molecular systematics provides an opportunity to revisit hypotheses raised by Fisher's earlier botanical and breeding studies on the alpine Ranunculi of New Zealand. Our work reported here describes molecular data and analyses that allow tests to be made between patterns of radiation, between hypotheses seeking to explain the geographic distribution of the group and whether speciation occurred following the onset of Pliocene mountain building in New Zealand. An amplified fragment length polymorphism (AFLP) method was used previously to find a highly variable DNA sequencing marker at the chloroplast J_{SA} region (the junction of the inverted repeat IR_A and small single-copy region SSC). The phylogenetic information from this marker has been compared in the present study

Table 2. General information about the voucher origin. N.Z.: New Zealand; S.Isl.: South Island; N.Isl: North Island; Mts.: Mountains; alp.: alpine; *R.*: *Ranunculus*.

Taxa	General distribution and habitat	Voucher, locality, collector	GenBank accessions ITS	GenBank accessions JSA
R. gunnianus	Australian Alps, wet tussock and grassland	MPN24587, Mt. Kosciusko, *T. Armstrong*	AF323298	AF323348
R. anemoneus	Australian Alps: Mt. Kosiusko, snow line fringe	MPN24588, Mt. Kosciusko, *T. Armstrong*	AF323273	AF323323
R. pinguis	Subantarctic Campbell and Auckland Islands, windswept sodden grassland and rocky moors	MPN24590, Campbell Island, *V. Nicholls*	AF323299	AF323349
		MPN24591, Auckland Island, *V. Nicholls*	AF323300	AF323350
R. sericophyllus	N.Z.: S.Isl., wet western southern alps, snowline fringe	MPN24595, Mt. Tutoko, *M. Steel*	AF323288	AF323338
		CHR529050, Lake Wapiti, *D. Glenny*	AF323289	AF323339
		CHR530524, Gertrude Valley: Black Lake, *D. Glenny*	AF323290	AF323340
		MPN24596, Mt. Cook, *M. Steel*	AF323291	AF323341
		MPN24597, Mt. Franklin, *M. Steel*	AF323292	AF323342
		MPN24667, Temple Basin, *M. Steel*	AF323293	AF323343
		MPN24668, Mt. Memphis, *D. Havell*	AF323294	AF323344
R. pachyrrhizus	N.Z.: S.Isl., drier eastern southern alps	MPN24598, Pisa Range, *D. Havell*	AF323295	AF323345
		MPN24599, Old Man Range, *A. Robertson*	AF323296	AF323346
R. viridis	N.Z.: Stewart Isl., granite ledges	No voucher (vulnerable/endangered), Mt. Allen, *D. Havell*	AF323297	AF323347
R. scrithalis	N.Z.: S.Isl., Eyre mountains, clay scree	MPN24600, Hummock Peak, *P. Lockhart*	AF323305	AF323355
R. lyallii	N.Z.: S.Isl., high rainfall areas on well-drained soils, gullies, shrubland, grassland	MPN24601, Mt. Tutoko, *M. Steel*	AF323283	AF323333
		MPN24602, Mt. Franklin, *M. Steel*	AF323274	AF323324
		MPN24603, Mt. Cook, *M. Steel*	AF323277	AF323327
		CHR 306134A, Mt. George, *D. Given*	AF323278	AF323328
		CHR110835, Hump Ridge, *E. J. Godley*	AF323282	AF323332
		MPN24669, Temple Basin, *M. Steel*	AF323275	AF323325
		MPN24670, Franz Josef, *D. Havell*	AF323276	AF323326
R. buchananii	N.Z.: S.Isl., southern alps, snowline fringe, scree	CHR509922, Garvie Mts.: above Skeleton Lake, *D. Glenny*	AF323280	AF323330
		CHR529051, Lake Wapiti, *D. Glenny*	AF323281	AF323331
		MPN24761, Takatimu Range: Clare Peak, *R. Havell*	AF323279	AF323329
R. haastii subsp. *haastii*	N.Z.: S.Isl., eastern alp. distribution, stone debris	CHR518452, Mt. Hutt, *P. Heehnan*	AF323284	AF323334
		CHR532284, Amuri Ski field, *D. Glenny*	AF323285	AF323335

Table 2. Continued.

Taxa	General distribution and habitat	Voucher, locality, collector	GenBank accessions ITS	GenBank accessions JSA
R. haastii subsp. *piliferus*	N.Z.: S.Isl., eastern alp. distribution, stone debris species	CHR509769, Eyre Mts., Helen Peaks, *D. Glenny*	AF323301	AF323351
		MPN24604, Eyre Mts.: Hummock Peak, *P. Lockhart*	AF323302	AF323352
R. grahamii	N.Z.: S.Isl., Mt. Cook region, rocky crevices and ledges above the permanent snowline	CHR217997, Ben Ohau Range: Twins Basin, *A. C. Archer*	AF323286	AF323336
		MPN24672, Malte Brun: Aiguilles Rouge, *M. Steel*	AF323287	AF323337
R. insignis	N.Z.: N.Isl. and central–northern S.Isl., sheltered situations	MPN24605, S.Isl.: Foggy Peak, *D. Glenny*	AF323306	AF323356
		MPN24606, N.Isl.: Volcanic Plateau, *D. Havell*	AF323307	AF323357
R. verticillatus	N.Z.: southern N.Isl. and central– northern S.Isl., grasslands	MPN24607, N.Isl.: Mt Holdsworth, *D. Havell*	AF323303	AF323353
		CHR530493, S.Isl.: Cobb Valley: Mt. Mytton, *D. Glenny*	AF323304	AF323354
R. nivicola	N.Z.: N.Isl.: Volcanic Plateau to Mt. Hikurangi, generally higher altitudes than *R. insignis* and *R. verticillatus*, grassland and stone debris	MPN24608, Volcanic Plateau, *P. Lockhart*	AF323308	AF323358
R. godleyanus	N.Z.: central S.Isl., rocky places at the snowline fringe	CHR499398, Havelock River: Eric Sream, *M. Harding*	AF323309	AF323359
		MPN24609, Malte Brun, *M. Steel*	AF323310	AF323360
R. crithmifolius subsp. *paucifolius*	N.Z.: S.Isl., Castle Hill only, limestone fine scree	No voucher (endangered), Castle Hill, *P. Lockhart*	AF323312	AF323362
R. crithmifolius subsp. *crithmifolius*	N.Z.: S.Isl., stony debris	CHR509774, Eyre Mts: Shepherd Saddle, *D. Glenny*	AF323311	AF323361
		MPN24673, Eyre Mts.: Big Jungle Creek, *B. Rance*	AF323313	AF323363
R. enysii	N.Z.: S.Isl., eastern alp. distribution, sheltered situations	CHR509805, Clarence River: Island Pass, *D. Glenny*	AF323316	AF323366
		MPN24610, Nevis Valley, *B. Rance*	AF323317	AF323367
		MPN24611, Umbrella Mts., *B. Rance*	AF323318	AF323368
R. gracilipes	N.Z.: S.Isl., damp and tussock grassland, scrub and sheltered rocky places	CHR529048, Remarkables: Lake Alta, *D. Glenny*	AF323314	AF323364
		MPN24612, Eyre Mts.: Symmetry Peaks, *P. Lockhart*	AF323315	AF323365
R. acaulis	N.Z., subantarctic Islands, Australia, Southern Chile, coastal habitats	MPN24592, Stewart Isl.: Masons Bay, *D. Havell*	AF323319	-
R. sceleratus	Europe, North America, spread worldwide	MPN24589, Germany: Oberpfalz, *H. Lehmann*	AF323322	-
R. circinatus	Eurasia, aquatic habitats	MPN24674, Germany: Franken, *H. Lehmann*	AF323321	-
R. recens	N.Z., lowland coastal, local populations	No voucher (endangered), Stewart Isl.: Masons Bay, *D. Havell*	AF323320	-

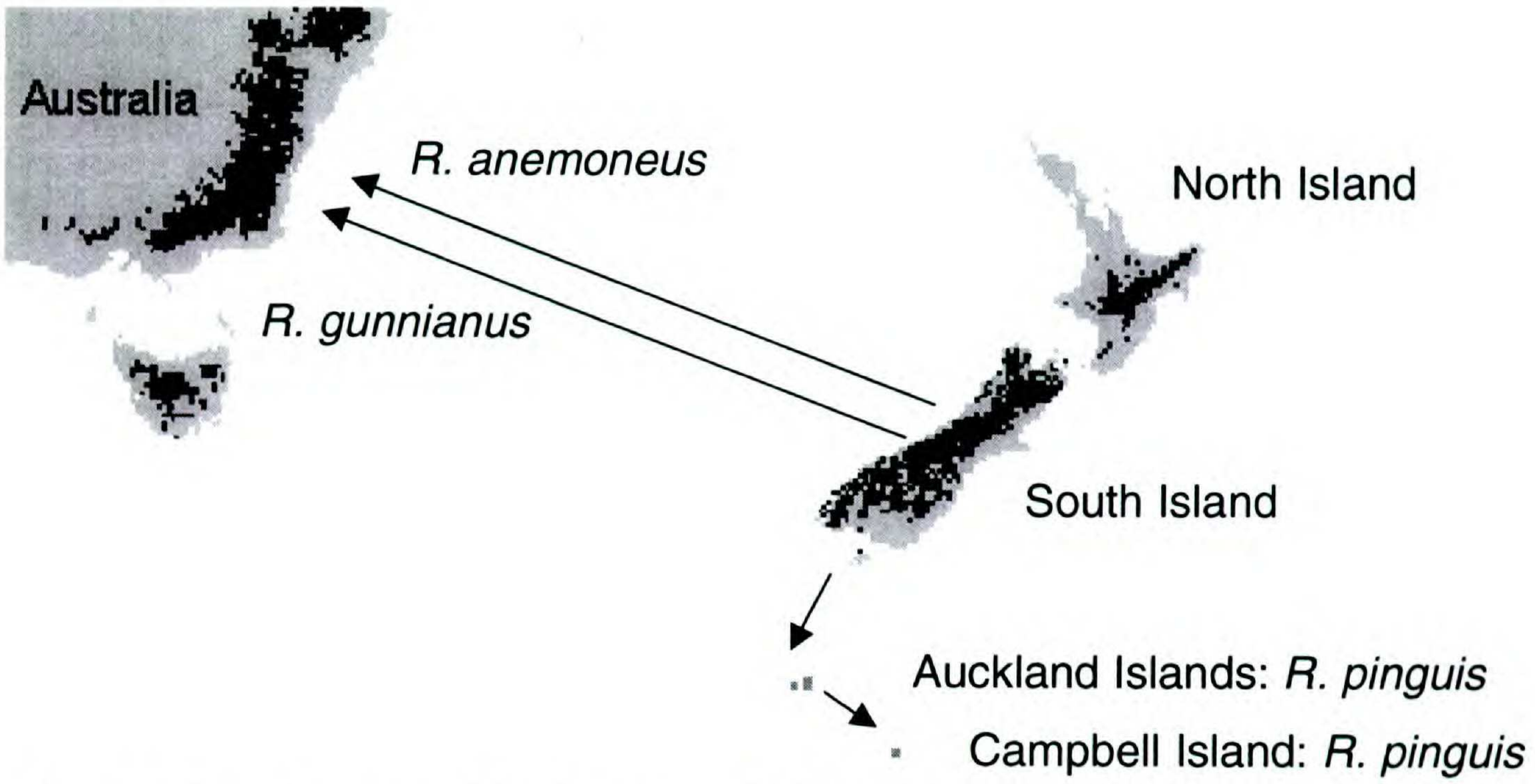


Figure 1. Geographic context of the alpine Ranunculi of New Zealand. The species labeled are those that have arisen following transoceanic dispersal from New Zealand in the directions indicated by arrows.

with that of the commonly used nuclear ITS marker (Johnson & Soltis, 1995). We have studied these data under quartet puzzling (Strimmer & von Haeseler, 1996) and split decomposition (Bandelt & Dress, 1992; Huson, 1998). The first method was used to obtain a rooted phylogenetic and graphic representation of the data. We used split decomposition to investigate more closely the phylogenetic relationships within two monophyletic groups from the New Zealand alpine radiation.

Materials and Methods

Plant material, distributions, and geography

Table 1 provides the authorities and references for the ingroup and outgroup species studied in the present work. *Ranunculus acaulis*, *R. sceleratus*, and *R. circinatus* were selected as outgroups based on the results of an earlier molecular study (Johansson, 1998). Table 2 gives details of the habitats, collection sites, names of collectors, herbarium vouchers, and GENBANK accession numbers. Vouchers for accessions are held at the Massey University herbarium (MPN) and the Landcare herbarium, Lincoln (CHR). The geographic context of our study is shown in Figure 1. The New Zealand distributions of alpine *Ranunculus* species are shown in Figure 2.

Identifying hypervariable regions in plant genomes

Experimental protocols that examine restriction endonuclease site differences between plant genomes can provide a helpful means to detect rapidly evolving plant genome regions. In a preliminary study we used the AFLP method of Vos et al. (1995) with AT-rich selective primers in an attempt to identify rapidly evolving length mutations (Golenberg et al., 1993) and restriction site differences in the chloroplast and mitochondrial DNAs of New Zealand alpine Ranunculi (Lockhart & McLenachan, 1997). Length mutations can occur due to the presence of small monomeric and direct repetitive sequences (Powell et al., 1995; Golenberg et al., 1993) and appear in genome regions that show elevated substitution rates. They have been found in chloroplast genomes at sites of intramolecular recombination such as in the single copy regions (at tRNA and *acc*D loci: Maier et al., 1995; Yasui & Ohnishi, 1998), and at the junctions of inverted repeat/single copy regions. These include those at the J_{LA} region: *mat*K-*psb*A (Powell et al., 1995; Johnson & Soltis, 1995), J_{LB} region: the *rpl*16 intron (Kelchner & Clark, 1997), and the J_{SB} region: *ndh*F (Olmstead & Sweere, 1994). Regions with direct repeats and elevated substitution rates have also been reported in DNAs of mitochondrial origin (e.g., Luo & Boutry, 1995).

One polymorphic region identified in our *Ranunculus* AFLP profiles was the chloroplast J_{SA} region (Lockhart & McLenachan, 1997). This locus appears to be previously unstudied in molecular systematic sequencing studies. Based on DNA sequence determined for a cloned AFLP profile band, we designed PCR primers (5′ATTATYAATGAAGGYAATACWATATATTTTC 3′ and 5′CAAATTCCAATGAC-

CAAATAGTTCG 3′) with which, using a standard PCR thermocycling profile (94°C 2 min., followed by 35 cycles: 94°C 30 secs., 50°C 1 min., 72°C 1 min., 72°C 5 min.), we amplified a portion of the J_{SA} region in species of New Zealand alpine Ranunculi. Due to the presence of direct repeats in the sequences of some taxa, this fragment ranged in size from 493 to 511 bp. We also amplified and sequenced 598–603 bp of the nuclear ITS (ITS 1, 5.8S rDNA, ITS 2) region using standard primers (from Baldwin et al., 1995). For both J_{SA} and ITS regions, sequencing was done for multiple accessions of most New Zealand alpine species. Characterization of PCR products was made for both DNA strands using an ABI377 sequence protocol. Aligned sequences have been submitted to GENBANK and are available with the accession numbers AF323323–AF323368; AF323273–AF323308. More recently we have also used AFLP to find molecular markers for polymorphic genome regions in *Nothofagus* (Nothofagaceae), *Myrsine* (Myrsinaceae), *Rhopalostylis* (Palmae), *Myosotis* (Boraginaceae), and *Phormium* (Phormiaceae) (McLenachan et al., 2000).

Phylogenetic Reconstruction and Plant Speciation

quartet puzzling

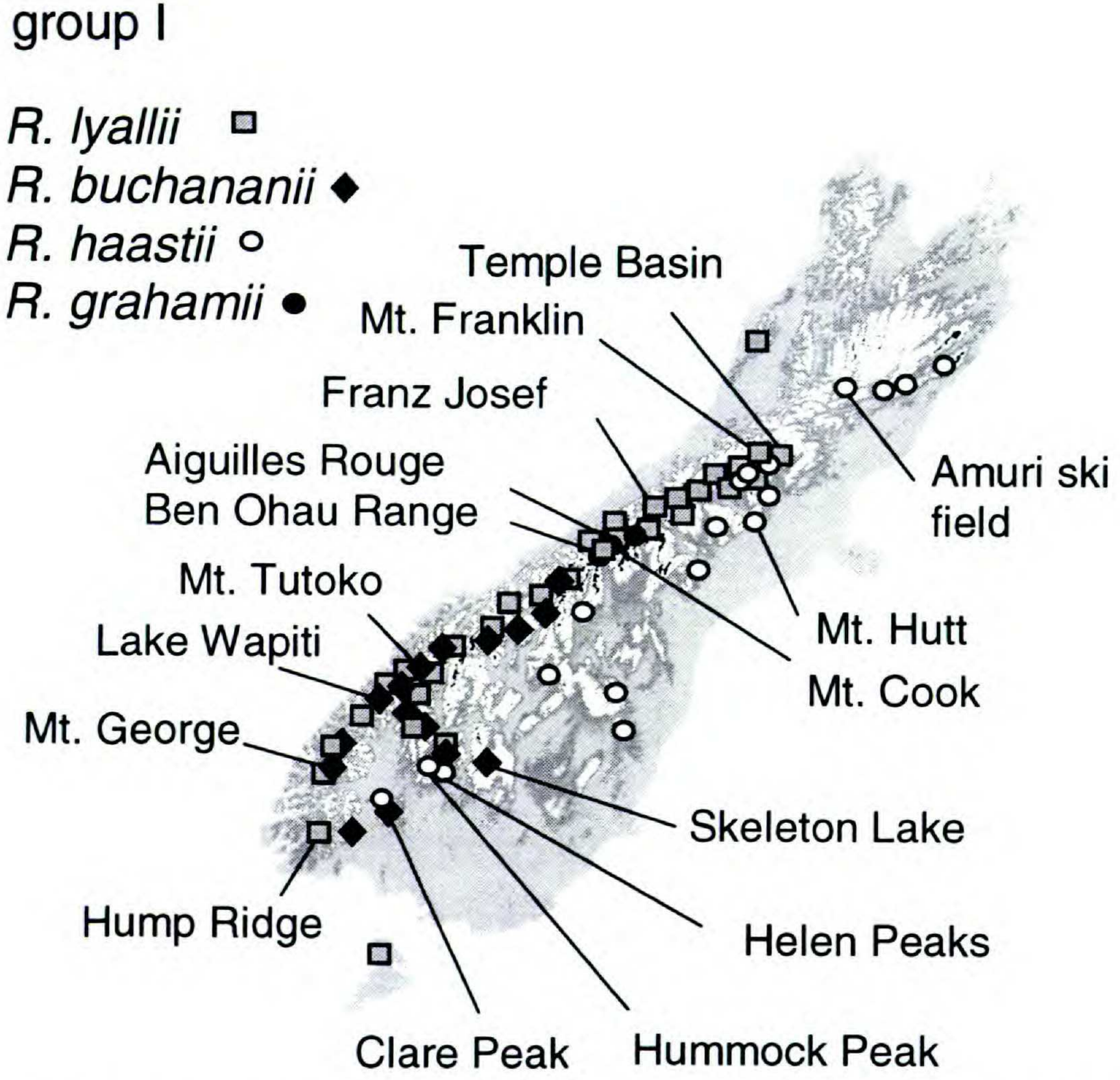
Quartet puzzling (QP, Strimmer & von Haeseler, 1996) was used to represent the phylogenetic structure of the main groups and lineages within the New Zealand alpine radiation. Evolutionary trees were reconstructed using the implementation of QP in PAUP*4.0b3a (Swofford, 1998). With the ITS data, outgroups were used to indicate the direction of evolution within the graph. Support for quartets was evaluated under minimum evolution criteria (Swofford et al., 1996) using observed distances that included insertion and deletion characters. With the ITS data, heteroplasmic sites were removed before tree building. QP is a heuristic tree building procedure that works efficiently (Penny et al., 1996) with a large number of taxa, to give a bifurcating tree onto which edge (branch) lengths can then be estimated. In QP phylogenetic trees, the support values assigned to groupings of taxa are usually interpreted as similar to bootstrap values (Strimmer & von Haeseler, 1996). However, they can differ considerably from bootstrap values in the case of recently diverged sequences, such as in the study of Late Tertiary–Quaternary plant radiations. Unlike bootstrap values, QP values are usually high when sequence data show few informative sites but relatively few incompatibilities. The problem of interpreting low bootstrap values in molecular studies of recently evolved taxa has been noted elsewhere (Knox & Palmer, 1995; Bandelt et al., 1995). QP values provided us with an indication in our molecular data of the degree of compatibility for phylogenetic groupings.

a problem with bifurcating evolutionary tree models

Phylogenetic analysis of DNA sequences from Late Tertiary–Quaternary plant radiations can be complicated when molecular markers showing suitable levels of variation exist at multiple loci in plant genomes (e.g., Buckler et al., 1997; Shan et al., 1999; Linder et al., 2000; McLenachan et al., 2000). When these sequences show differences from each other, bifurcating evolutionary tree models (such as commonly implemented under parsimony, neighbor joining, and maximum likelihood selection criteria) usually provide a poor representation of the phylogenetic complexity of the sequence information (e.g., Sang et al., 1994; Koch & Al-Shehbaz, 2000). These more commonly used approaches to tree building also provide poor representation when extant taxa are characterized by sequences that have not diverged from those of ancestors. In this case, commonly used methods tend to force taxa away from internal points in the tree and onto bifurcating tips of the reconstructed tree.

Split Decomposition

There seems to exist no simple solution to the problem of fully representing the phylogenetic information in sequence data and at the same time maintaining a low order of computational complexity in analysis. However, split decomposition (Bandelt & Dress, 1992; Huson, 1998) is one efficient approach that provides a means to investigate and represent more complex substitution patterns in an alignment of sequences (e.g., Lockhart et al., 1995). Presently implemented under parsimony or distance criteria (Huson, 1998), it allows investigation of whether the data structure is or is not tree-like and bifurcating. If sequences show evidence of recombination this will be represented in the form of reticulate graphs. In the present study we used the implemention of split decomposition in SplitsTree 3.1 (Huson, 1998) to examine the relationships between taxa belonging to two lineages within the New Zealand alpine radiation. The relationships within a third New Zealand alpine lineage have been studied elsewhere (Huber et al., 2001) using median and pruned median networks—which are methods also seeking to more fully represent the



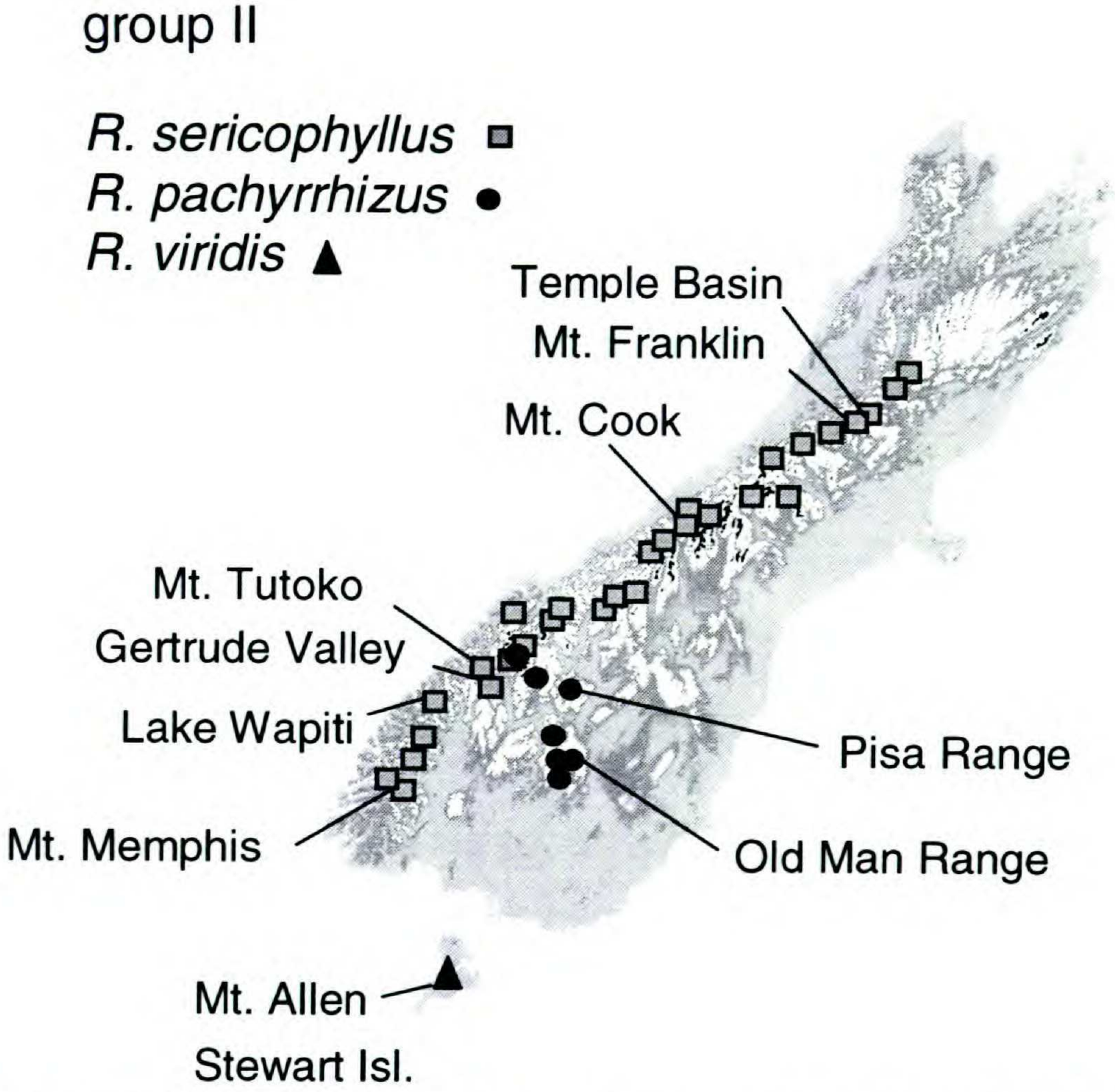
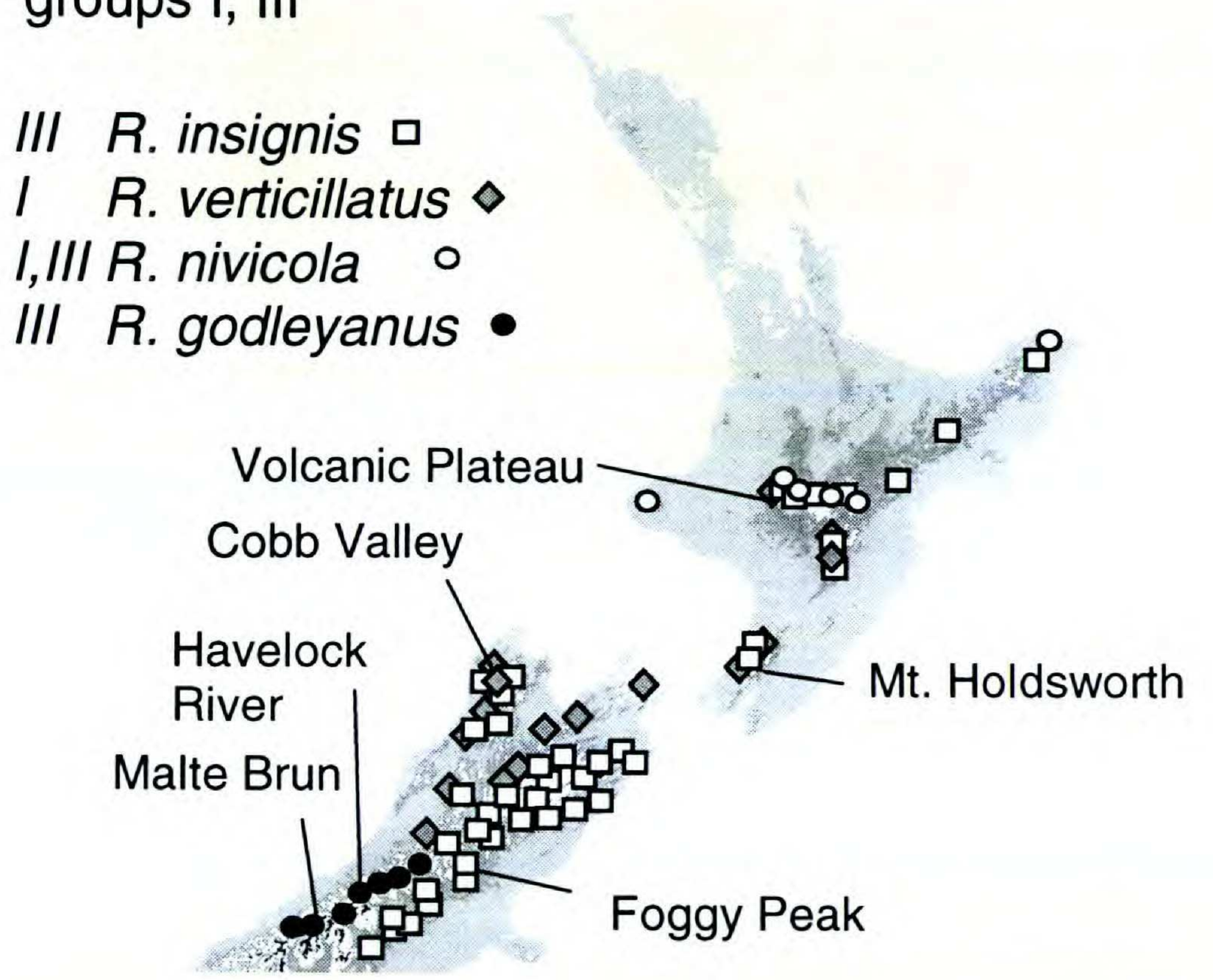


Figure 2. Distributions of taxa from different phylogenetic groupings (I–IV) in the North, South, and Stewart Islands. Sites of known collections are indicated by symbols. These locations are reported in Fisher (1965), Webb et al. (1988), and identified in our own collections. The sites at which sample collections were made in the present study are indicated by names.



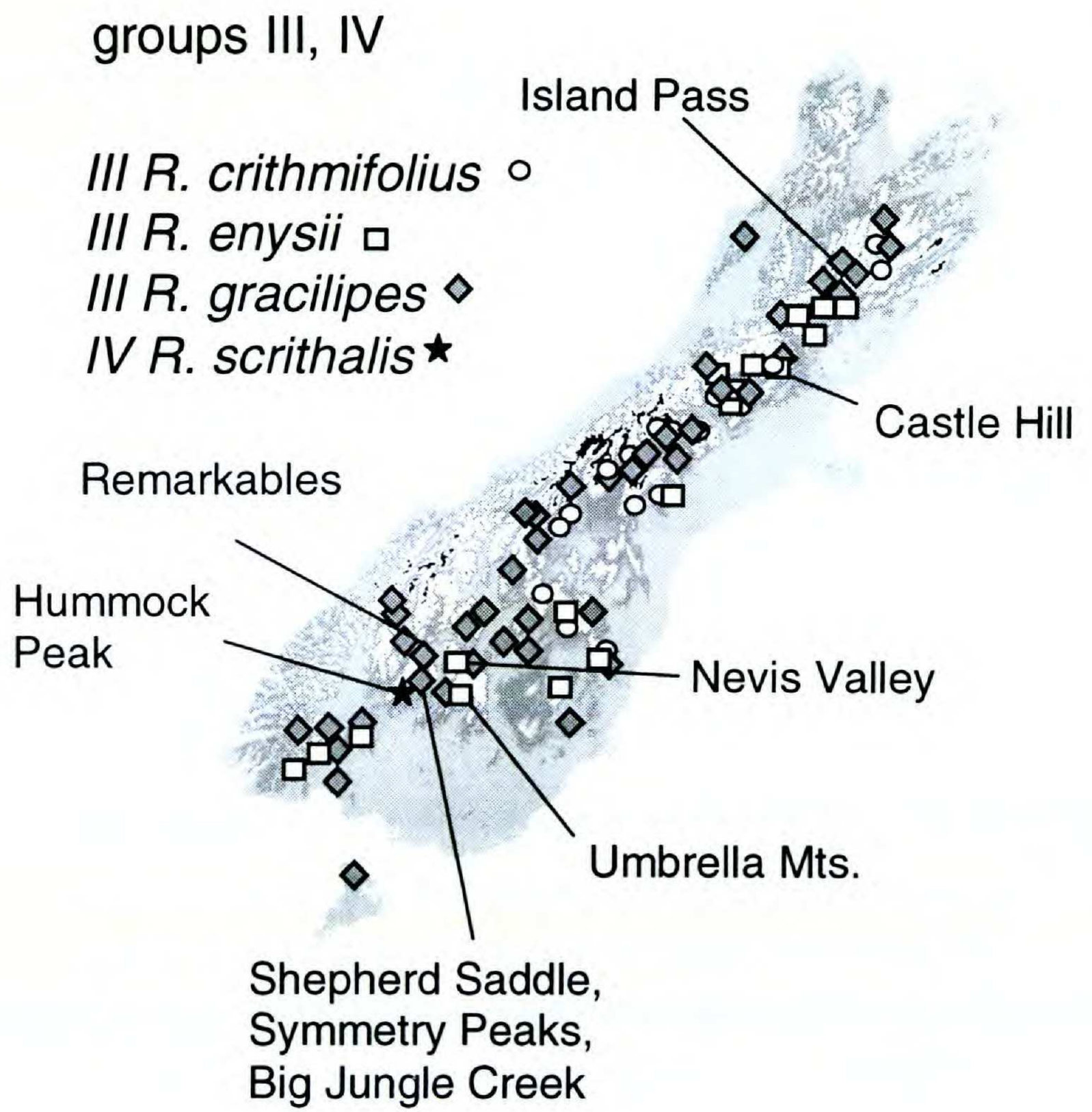


Figure 2. Continued.

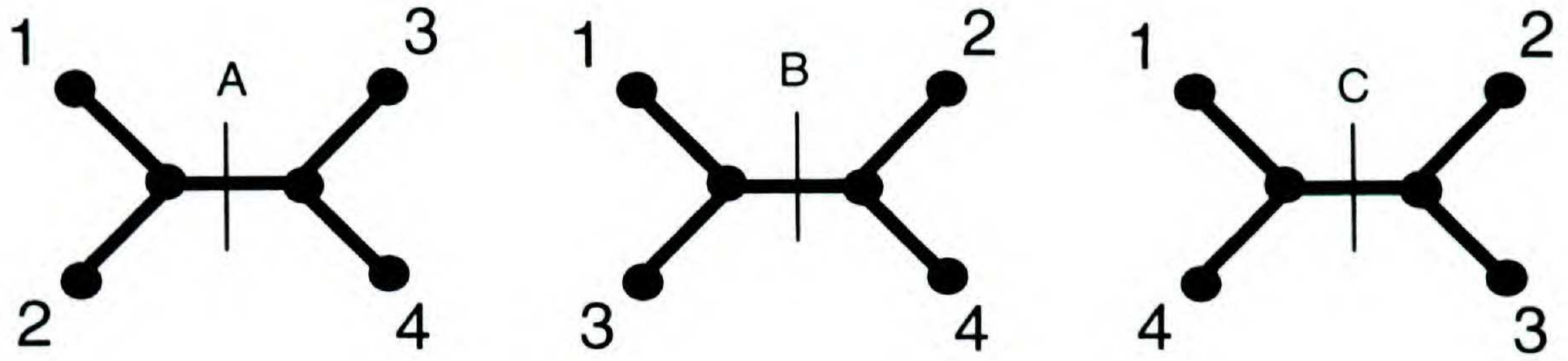


Figure 3. The splits A, B, and C uniquely identify the three possible quartets for the taxa 1, 2, 3, 4.

phylogenetic complexity of sequence data. We did not include insertions and deletions in our splits graphs, which were calculated from observed distances. These indels were all compatible with splits in the splits graphs shown and have been indicated in the figure legends. Since split decomposition has rarely been used by plant systematists (but see Bandelt, 1995; Koch & Al-Shehbaz, 2000), we provide a simple description of the approach. The procedure involves three distinct steps:

(A) FINDING WEAKLY COMPATIBLE SPLITS

First, all possible combinations of four taxa from a data set (perhaps containing many taxa) are examined. For each combination of four taxa, support is evaluated for each of the internal "splits" that uniquely identifies the possible bifurcating trees (for every combination of four taxa, three bifurcating trees are possible: Fig. 3).

Parsimony, distance, or maximum likelihood (or other) criteria can be used in deciding which two of the three bifurcating trees are most strongly supported by the data. Here, we use a distance calculation to obtain measures of support (isolation index values). For split A (Tree 1) the isolation index $= 0.5 \times (d_{14} + d_{23} - d_{12} - d_{34}) = 0.5 \times (d_{24} + d_{13} - d_{12} - d_{34})$; for tree split B (Tree 2) the isolation index $= 0.5 \times (d_{14} + d_{23} - d_{13} - d_{24})$; and for tree split C (Tree 3) the isolation index $= 0.5 \times (d_{13} + d_{24} - d_{14} - d_{23})$ where d is the path length between the pairs of taxa (e.g., perhaps the observed number of differences between the taxa). The two splits that have the highest isolation index score for each combination of four taxa are considered to be weakly compatible, and they are used to next obtain a split system that describes the relationship between *all* taxa in the original data set.

(B) OBTAINING A SPLIT SYSTEM

The weakly compatible splits from the quartet study define the presence of the internal splits that will also occur in the split system for all taxa in the data set. Since some of the quartets not excluded in the first step of the quartet study identify the *same* split between more than four taxa, a decision needs to be made as to which isolation index value will be used. For example, consider one particular split in a split system (as shown in Fig. 4). This split (A) separates taxa 1 and 2 from taxa 3, 4, and 5. The split is equivalently identified by the quartet splits: taxa 1 and 2 split from taxa 3 and 4; taxa 1 and 2 split from taxa 3 and 5; taxa 1 and 2 split from taxa 4 and 5; etc. In its standard implementation split decomposition chooses the *smallest* isolation index value from all the quartet splits that are compatible with that split in the split

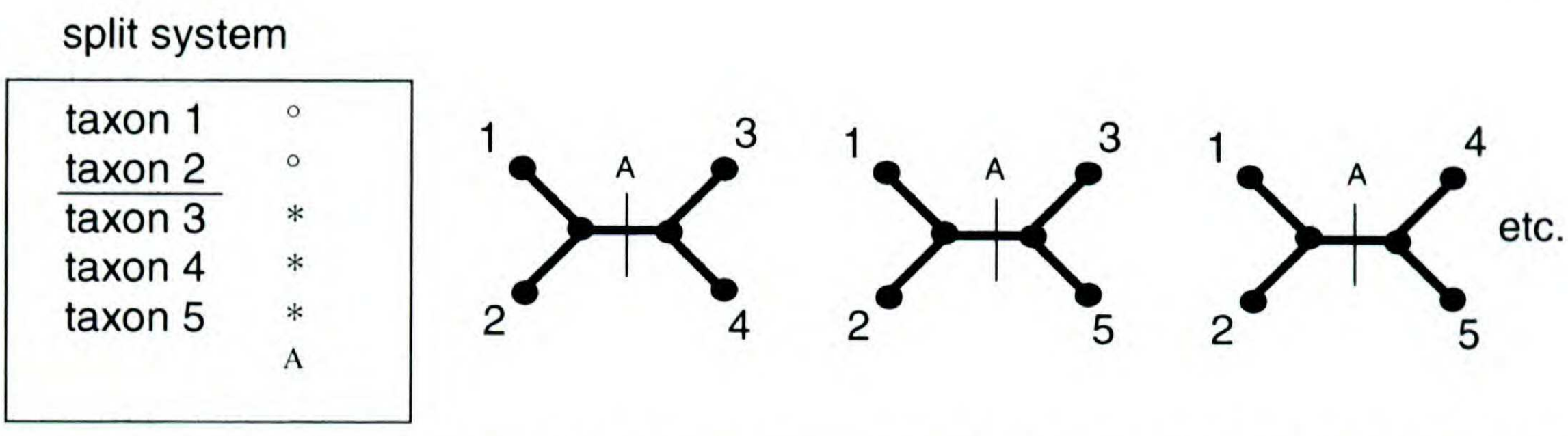


Figure 4. In the split system shown, split A separates taxon 1 and 2 from taxa 3, 4, and 5. Symbols (○, .) have been used to identify the taxa on either side of split A. This symbol representation is also used to identify the different splits in the split system shown in Figure 5.

system. The rationale for choosing the smallest value is that the method will be conservative in deciding whether there is really support for such a split in the split system. An alternative or less conservative approach is to average across values for compatible quartet splits (Moulton et al., 1997; Huson, 1998).

The split system must also contain the splits that correspond to the external edges or branches in the final tree or network. Support for these is calculated using all combinations of three taxa. If such taxa are identified as i, j, k then the isolation index for the split (edge) leading to $i = 0.5 \times (d_{ij} + d_{ik} - d_{jk})$. Split decomposition chooses the smallest value for the length of that split.

(C) BUILDING A SPLITS GRAPH

Once the splits present in a split system have been identified and their values assigned, a graph is constructed from the split system by an algorithm that splits taxa from each other (e.g., see Fig. 5).

If the split system contains no incompatibilities (as in split system i, or in the more resolved split system ii), a tree-like structure will result. If there are incompatibilities a network (boxes) will appear (split system iii). In this graph, the edges (internodes) have lengths that correspond to the isolation index values of the splits in the split system. In some cases, these lengths will be an approximation of those in the distance matrix, in which case a lower fit statistic is associated with the graph (the fit statistic = sum of all the paths in the graph divided by the sum of all paths in the pairwise distance matrix). In splits graphs, genotypes are not forced onto the tips of bifurcating trees. Thus, splits graphs provide more informative graphic representations when sequences from the accessions examined are identical to those in ancestors. Splits graphs are also advantageous in that they highlight the strongest incompatible signals in data that indicate contradictory evidence for cladistic relationships.

A limitation with the current implementation of split decomposition is the underestimation of internal edge lengths when the number of taxa compared is large and when the divergence between some taxa is great. In the present study we used split decomposition to study very closely related taxa. In our example, the split decomposition fit statistic was always very high, indicating that our splits graphs well represented the information contained in the sequences studied.

Molecular Clock Analyses

We used the method of Steel et al. (1996) as implemented in Splitstree 3.1 (Huson, 1998) to examine the molecular clock-like properties of ITS1 and ITS2 sequences for the two alpine *Ranunculus* breeding groups identified by Fisher (1965). This relative rates test compares the sequence differences between three taxa and examines whether the path lengths across the internal node that separates two of the taxa from the third are statistically equal. In the present study this investigation was made for all combinations of three taxa in our data matrix. To estimate the genetic distance (and subsequently time of divergence) between the two groups we also used a method suggested by Steel et al. (1996) implemented in Splitstree 3.1 (Huson, 1998). This procedure makes use of all available sequence data, within two groups, to help reduce the variance on the estimate of divergence between those two groups. Our estimate for the number of substitutions per site per million years was made with a Jukes-Cantor substitution model (Swofford et al., 1996) and compared against calibrated divergences of ITS1 and ITS2 sequences from an earlier study on *Dendroseris* (Sang et al., 1994). This comparison allowed us to obtain a tentative estimate for the divergence time of the two breeding groups.

Results

FOUR ALPINE GROUPS

Quartet puzzle phylogenetic trees reconstructed from our ITS (ingroup and outgroup taxa) and J_{SA} (ingroup taxa) sequences are shown in Figure 6 and Figure 7, respectively. These results indicate that the alpine Ranunculi of New Zealand (species from within sect. *Pseudadonis*) comprise four distinct phylogenetic groups. *Group I* includes alpine species occupying (i) habitats at the snowline fringe: *R. anemoneus* (Australian alps) and *R. buchananii* (New Zealand Southern alps), (ii) wet well-drained habitats: *R. lyallii*, (iii) stony screes: *R. haastii* subsp. *haastii* and *R. haastii* subsp. *piliferus* (New Zealand Southern alps), (iv) habitats above the permanent snowline: *R. grahamii*, and (v) poorly drained alpine shrub and tussock land: *R. verticillatus* (but note that the chloroplast data suggest that *R. verticillatus* is distinct from this group). *Group II* comprises alpine and subantarctic species: the Australian alpine species *R. gunnianus*, the New Zealand snowline fringe species *R. sericophyllus* and *R. pachyrrhizus*, *R. viridis* from Mt. Allen on Stewart Island, and the subantarctic *R. pinguis* from Campbell and Auckland Islands. *Group III* contains

split system i

taxon 1	oo
taxon 2	oo
taxon 3	oo
taxon 4	*o
taxon 5	**
taxon 6	**
	AB

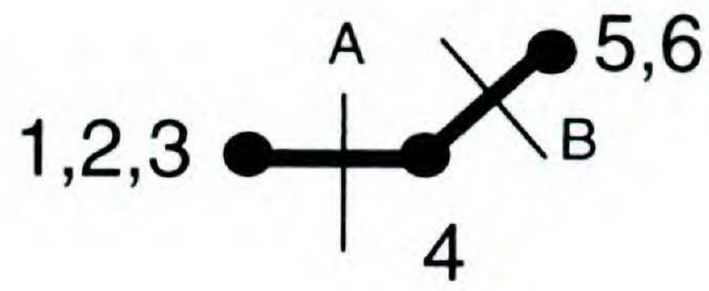
split system ii

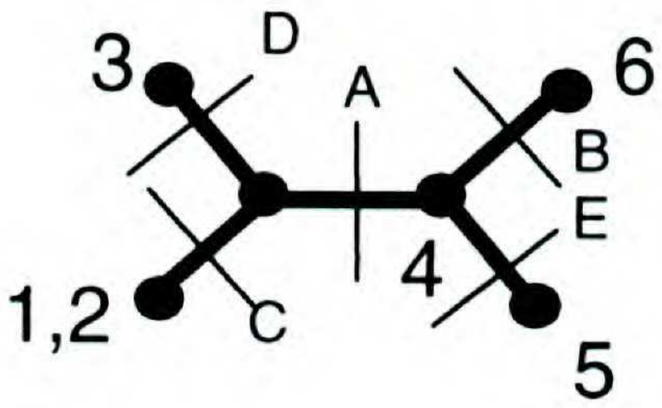
taxon 1	ooo**
taxon 2	ooo**
taxon 3	oo*o*
taxon 4	*o***
taxon 5	*o**o
taxon 6	*****
	ABCDE

split system iii

taxon 1	ooo**o
taxon 2	ooo**o
taxon 3	oo*o**
taxon 4	*o***o
taxon 5	*o**oo
taxon 6	******
	ABCDEF

splits graphs





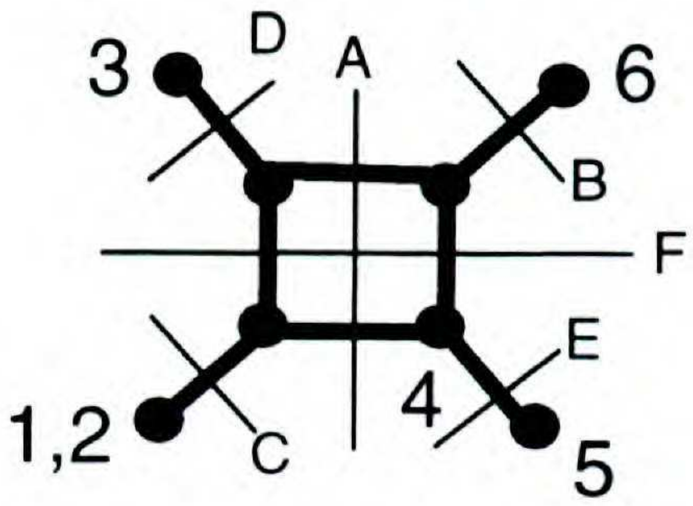


Figure 5. Examples of split systems and splits graphs. The splits (but not their lengths) in the split systems on the left have been used to construct the figures on the right.

alpine species having only a New Zealand distribution. These include both subspecies of the restricted scree specialist *R. crithmifolius*, the snowline fringe species *R. godleyanus*, and species occupying lower altitude sheltered situations: *R. insignis*, *R. enysii*, and *R. gracilipes*. *Group IV* is represented by a single alpine species, the scree specialist *R. scrithalis* from the Eyre mountains, South Island, New Zealand. The groupings in our trees are comparable with Fisher's (1965) two main phylogenetic groups, i.e., our groups I and II are Fisher's *many petals, silky hair* group, while group III

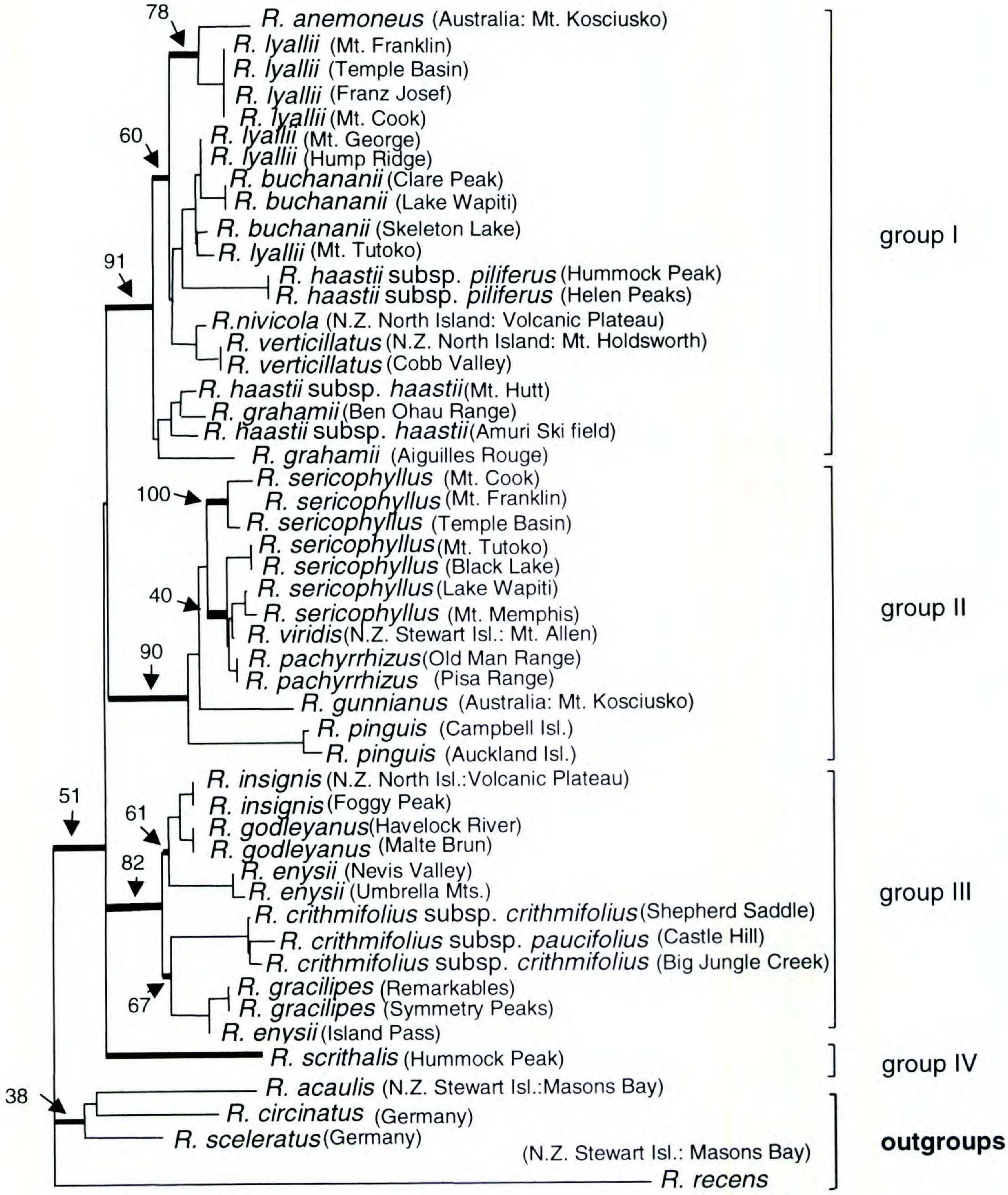


Figure 6. Quartet Puzzle tree showing branch lengths for ingroup and outgroup nuclear ITS sequences. Splits identifying groups I–IV are indicated by bold lines. The Quartet Puzzle support values for these and other splits discussed in the text have been shown. Unless indicated otherwise, taxa were sampled from the South Island of New Zealand.

identifies Fisher's *few petals, coarse hair* group. Group IV, represented by *R. scrithalis*, was not studied by Fisher.

In both the ITS and J_{SA} graphs, groups I–III are separated from each other by moderate to high QP support values. In the ITS graph, which contains outgroup species to root the tree, the relationships between the four groups and outgroups is unresolved, and visually would be represented as a star phylogeny in an unrooted phylogram. There is no evidence in the ITS data to suggest that the outgroups should join with either of the Australian

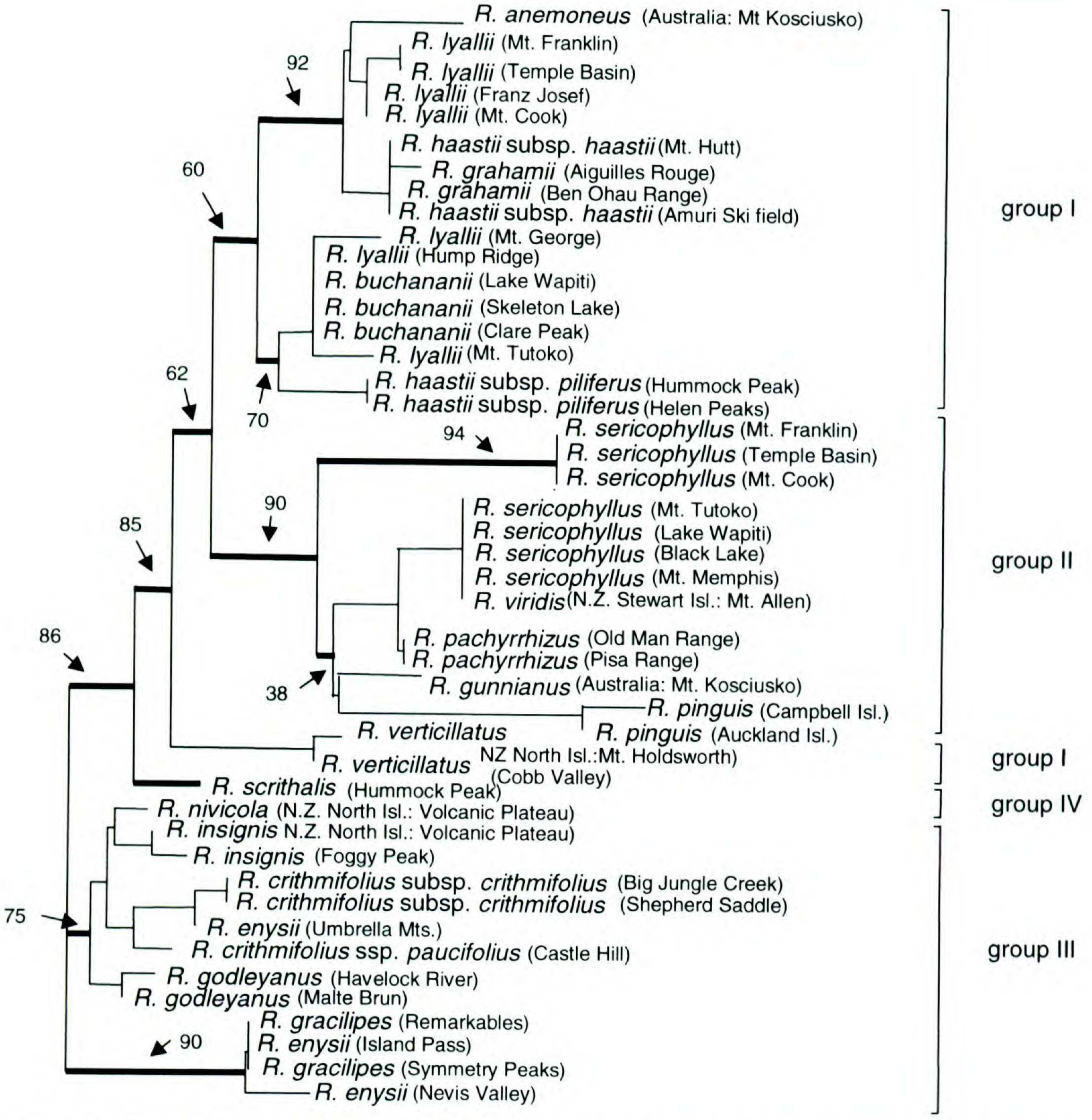


Figure 7. Quartet Puzzle tree for ingroup J_{SA} sequences. Details are the same as described in the legend for Figure 6.

species (0% QP values for outgroups with *R. anemoneus*; 0.3% QP values for outgroups with *R. gunnianus*) or with the subantarctic *R. pinguis* (1.7% QP values for outgroups with *R. pinguis*). Such groupings would be expected if the Australian or subantarctic island species were ancestral to the New Zealand alpine species.

The molecular clock test of Steel et al. (1996) was used to study combined ITS1 and ITS2 data from accessions for taxa corresponding with Fisher's two breeding groups, i.e., *R. buchananii*, *R. haastii* subsp. *haastii*, *R. haastii* subsp. *piliferus*, *R. lyallii*, *R. pachyrrhizus*, *R. sericophyllus*, versus *R. crithmifolius* subsp. *crithmifolius*, *R. crithmifolius* subsp. *paucifolius*, *R. enysii*, *R. gracilipes*, *R. insignis*. All sequences passed the test for a clock-like rate. From these data we obtained a mean point estimate for the age of the last common ancestor for the two breeding groups at 5.01 million years ago (Mya) (i.e., $0.0398088 \pm 0.00664/2 \times 3.94 \pm 0.10$ exp-9 Mya).

PATTERNS OF DIVERSIFICATION WITHIN GROUPS I AND II

The data for groups I and II were examined in detail under split decomposition in Figures 8–11. Comparison of the splits graphs for the chloroplast

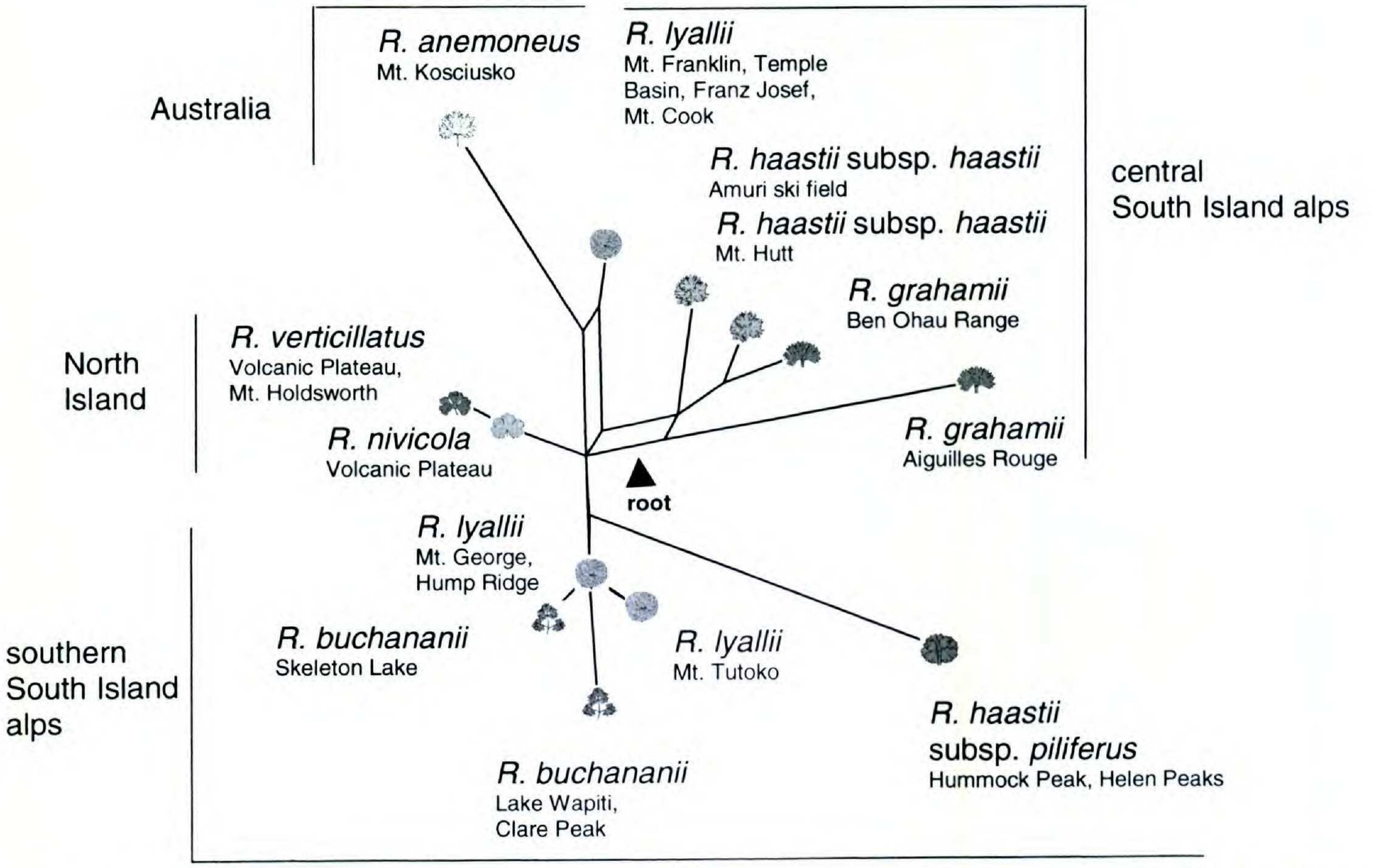


Figure 8. Splits graph of ITS sequences for taxa of group I. The inferred position of the root from Figure 6 is shown for reference. The fit of the splits graph to the ITS sequence data for taxa from group I is very high (Fit = 92).

and nuclear markers shows both tree-like (Fig. 9) and reticulate relationships (Figs. 8, 10, 11). Reticulation is evident between the chloroplast sequences from some of the most diverged species (Fig. 11) and between ITS sequences for some of the most closely related species (Fig. 10). In this latter instance, a complex network describes the relationship between sequences from southern South Island accessions of *R. sericophyllus*, *R. pachyrrhizus*, and *R. viridis* from Stewart Island. Multiple substitutions are one possible explanation for the occurrence of the reticulation between the more diverged chloroplast sequences. However, reticulation between the less diverged ITS sequences is more difficult to explain by the occurrence of multiple substitutions, and the pattern of reticulation may be indicative of hybrid relationships.

A feature of the splits graphs and quartet puzzle trees is that *R. lyallii* and *R. sericophyllus* are paraphyletic. These species have two centers of genetic diversity (i) in the central South Island and (ii) in the southern South Island alps.

Discussion

A monophyletic group

The analyses presented are consistent with the alpine Ranunculi of New Zealand (in the sense of Fisher, 1965; i.e., including the subantarctic *R. pinguis*) being a monophyletic group. This conclusion is supported by ITS sequencing for more widely sampled Australasian species (Armstrong, unpublished data). It is a well circumscribed group of taxa (Fisher, 1965; Webb et al., 1988) and represents section *Pseudadonis* (which includes 18 species; see Table 1). It is a predominantly alpine group, characterized by turgid achenes, simple nectaries, and disproportionately large flowers. Species are polyploid ($2n$ = 48, 96; Hair, 1983; Rendle & Murray, 1989) and linked by hybridization (Fisher, 1965).

A dispersed origin for the founding of an alpine lineage in New Zealand?

Our molecular clock estimates suggest that the primary divergence within the alpine Ranunculi of New Zealand (group III from groups I and II), referred to by Fisher (1965), occurred approximately 5 Mya: a result that would correlate with the onset of Late Tertiary mountain building in New Zealand (Cooper & Millener, 1993; Batt et al., 2000). Prior to this diversification, the evolutionary history of the alpine group is uncertain. The extent of genetic diversity between New Zealand alpine *Ranunculus* species and those from other Southern Hemisphere

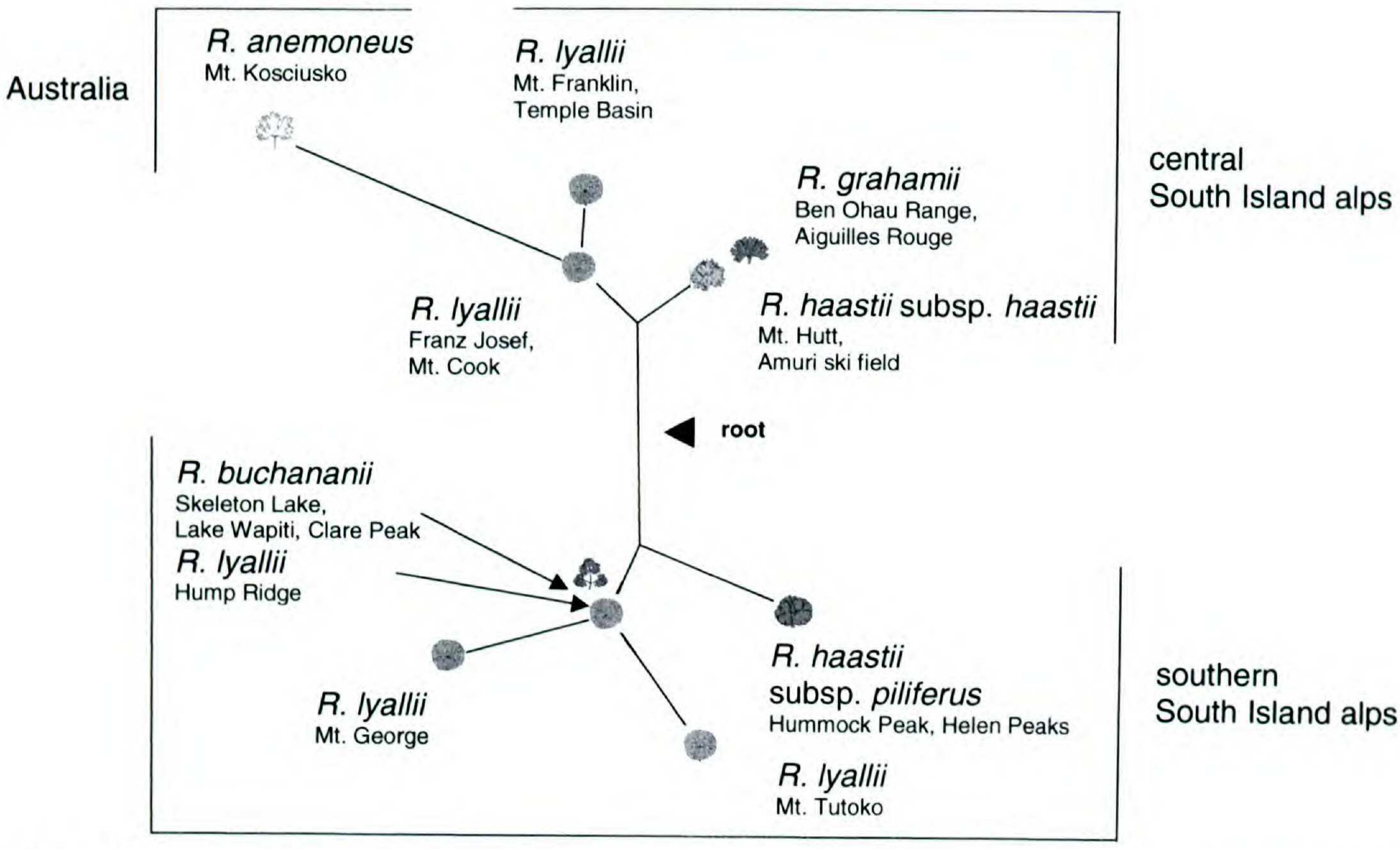


Figure 9. Splits graph of J_{SA} sequences for taxa of group I. The inferred position of the root from Figure 6 is shown for reference. A 9 bp deletion shared by all *R. buchananii* accessions and the *R. lyallii* accessions from Mt. George and Hump Ridge partitions these taxa away from the others in the data set. The fit of the splits graph to the J_{SA} sequence data for taxa from group I is very high (Fit = 100).

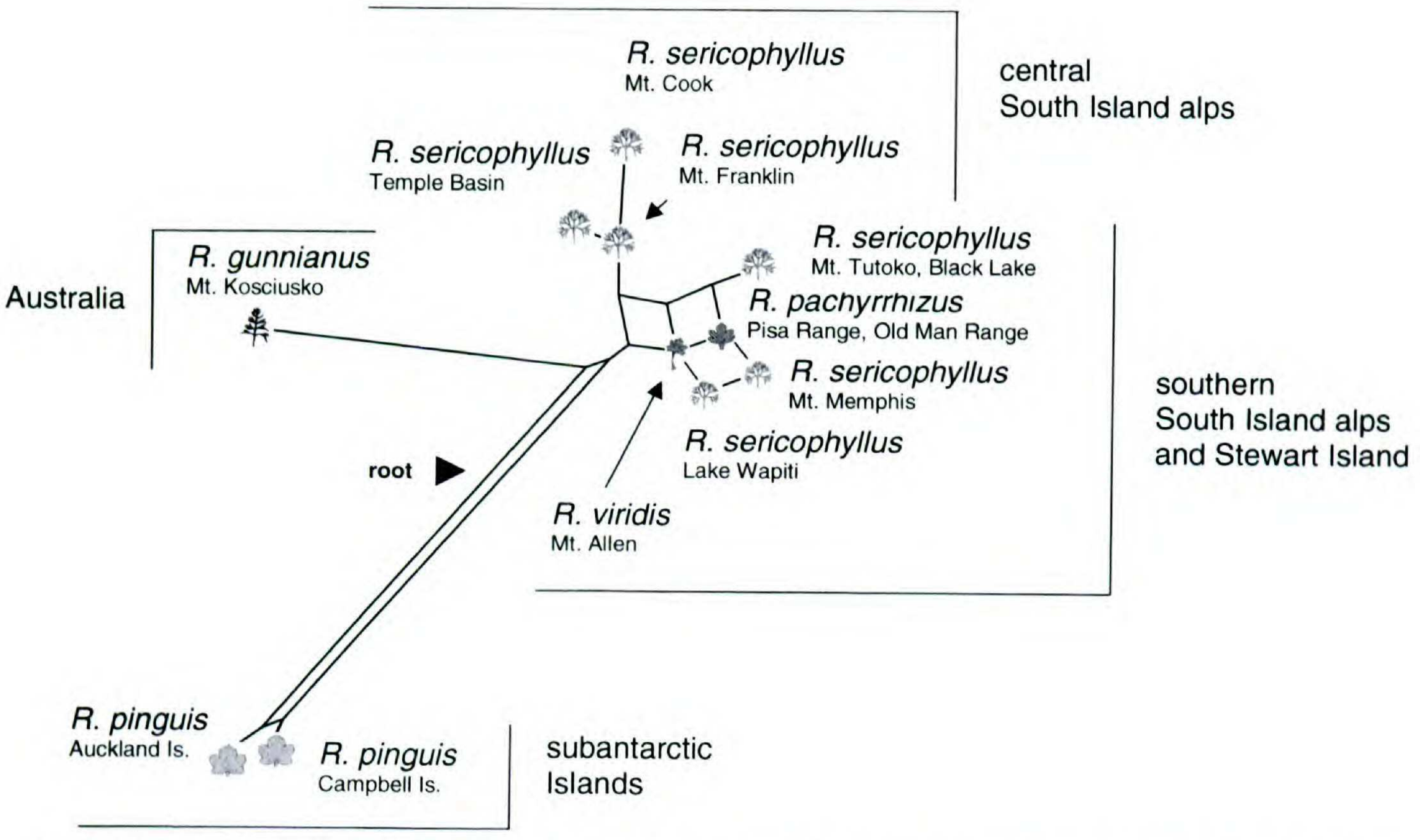


Figure 10. Splits graph of ITS sequences for taxa of group II. The inferred position of the root from Figure 6 is shown for reference. The fit of the splits graph to the ITS sequence data for species from group II is very high (Fit = 100).

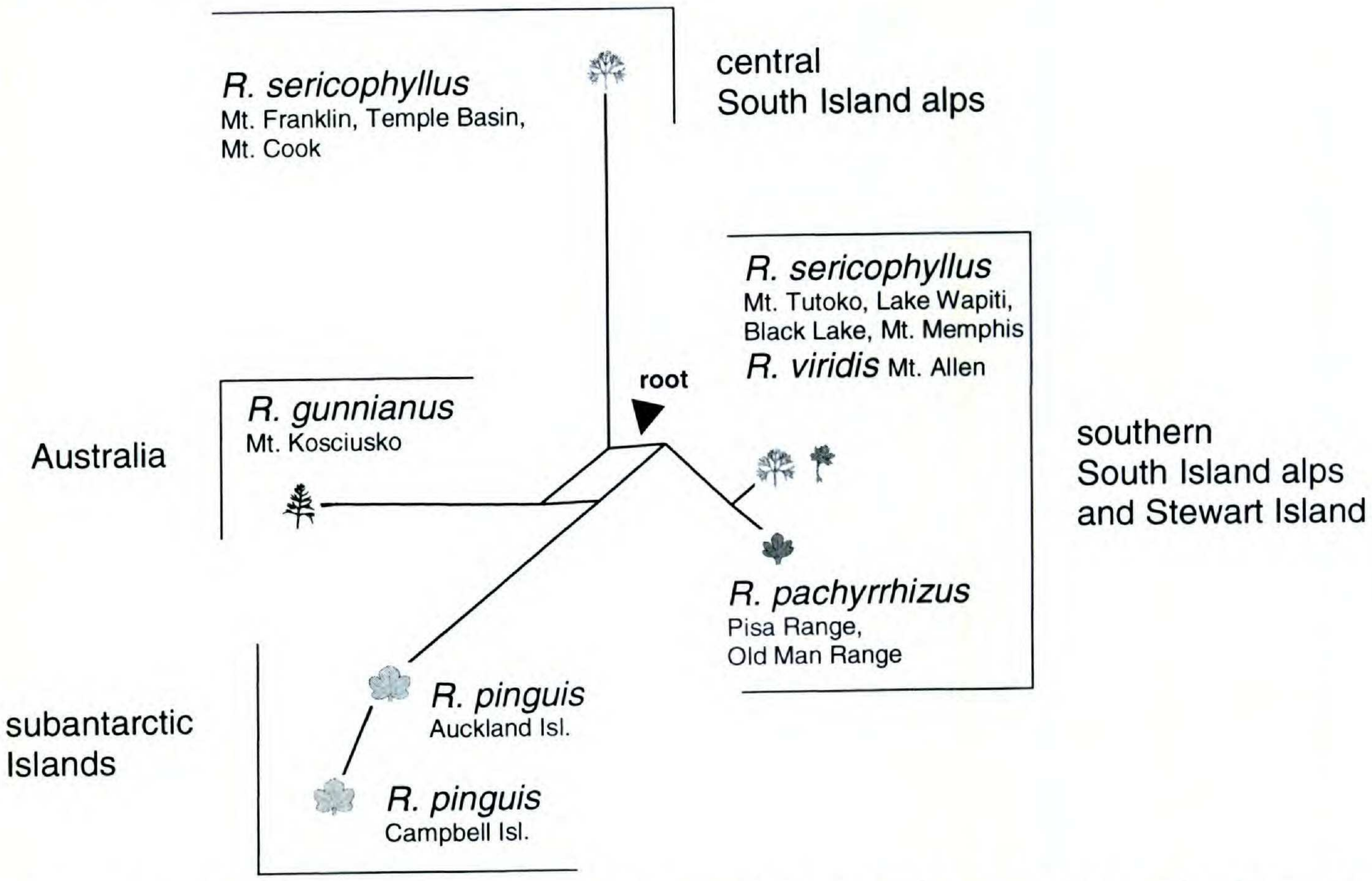


Figure 11. Splits graph of J_{SA} sequences for taxa of group II. The inferred position of the root from Figure 6 is shown for reference. A 9 bp insertion in *R. pinguis* partitions this species from the others in the data set. The fit of the splits graph to the J_{SA} sequence data for taxa from group II is very high (Fit = 100).

lands, as well as from those of the Northern Hemisphere, is too small to suggest that the group has an ancient relic (Gondwanan) distribution in New Zealand. Rather, it seems more likely that the group diversified following transoceanic dispersal of a founding species to New Zealand in the Tertiary.

Chloroplast restriction site data (Johansson, 1998) and unpublished ITS sequence data suggest that closely related to the alpine Ranunculi of New Zealand are worldwide distributed groups such as section *Hecatonia* (*R. sceleratus*), section *Xanthobatrachium* (*R. hyperboreus*), and subgenus *Batrachium* (*R. circinatus*). Two species from these groups were chosen as outgroups in our present study (Fig. 6). Also genetically close to the alpine Ranunculi in New Zealand are the Southeastern Australian species previously investigated by Melville (1955) and the lowland species of section *Epirotes*. These latter species do not appear to be included in section *Pseudadonis* by Tamura (1995), although Tamura does include from this group *R. rivularis* DC. (an earlier illegitimate name for *R. amphitrichus* Colenso: Webb et al., 1988). In Figure 6, this lowland predominantly Australian and New Zealandic group is represented by the coastal species *R. acaulis*. The New Zealand lowland Ranunculi from section *Chrysanthe* are represented here by *R. recens*. This group is more distantly related to the alpine Ranunculi of New Zealand.

It has been suggested (Fisher, 1965; Wardle, 1978; Zimmer & Keener, 1989) that the alpine Ranunculi of New Zealand are derived from South American species or at the least have closest affinity with extant alpine South American species, notably those of section *Trollianthoideae* Lourt. Like the New Zealand species, members of this group occupy alpine habitats and are characterized by disproportionately large flowers and turgid achenes (Fisher, 1965; Tamura, 1995; Lourteig, 1956). An alternative hypothesis has been suggested by Raven (1973). He proposed for many alpine plant genera, including *Ranunculus*, long-distance dispersal to New Zealand via the New Guinea archipelago and Australian mountains.

TRANSOCEANIC DISPERSALS FROM NEW ZEALAND

The hypothesis, that the ancestral alpine *Ranunculus* was originally dispersed to New Zealand, is consistent with the observations in our study that New Zealand has also been the source of transoceanic dispersals for alpine *Ranunculus* to other Southern Hemisphere lands. The Australian alpine

species *R. anemoneus* (strongly clustered within group I) and *R. gunnianus* (strongly clustered within group II) are genetically dissimilar from each other, and neither join with the outgroup species in phylogenetic analyses (e.g., Fig. 6). These observations suggest that the two Australian species are independently derived from within the New Zealand alpine radiation and are not ancestral to it. Similarly, the New Zealand subantarctic species *R. pinguis* (group II) also appears derived from within the New Zealand mainland radiation. In these three cases, dispersal from New Zealand has occurred against prevailing circumpolar westerly winds (Fig. 1), which have existed since the mid Tertiary (Stevens, 1985; Stewart & Neall, 1984). Nevertheless, in the southwestern Pacific frequent anticyclones give rise to easterly wind flows, and it is possible that these may have assisted dispersal in a westward direction (Wardle, 1978; Wallington, 2000).

LOCAL PATTERNS OF SPECIATION AND GLACIAL REFUGIA?

The splits graphs in Figures 8 and 9 indicate that, for group I species, the derivation of high-altitude species with highly restricted distributions has been from ancestral stock of the paraphyletic and geographically widespread *R. lyallii*. In the central South Island alps, i.e., in the northern part of its range (Fig. 2 upper left), *R. lyallii* ancestors appear to have given rise to higher-altitude species such as the scree specialists *R. haastii* subsp. *haastii* and *R. grahamii* of the Mt. Cook region (Figs. 2, 8, 9), both with yellow flowers and winged, curved, smooth achenes. In the southern South Island alps, (Fig. 2), the restricted high-altitude snowline fringe species *R. buchananii* is genetically most similar to southern South Island populations of *R. lyallii* and is inferred to be derived from southern *R. lyallii*-like ancestors (Figs. 8, 9). Note that the southern scree specialist *R. haastii* subsp. *piliferus* is not most closely related to *R. haastii* subsp. *haastii* (Figs. 8, 9), and these taxa presently designated as subspecies may not be monophyletic.

Somewhat in parallel, the splits graphs in Figures 10 and 11 indicate that the derivation of two group II species with highly restricted geographical distributions has been from ancestral stock of the paraphyletic and geographically widespread *R. sericophyllus*. This morphologically and genetically variable species is found along the high elevated ridges of the southern alps from north Canterbury to southwest Otago. The relationships observed in these graphs are consistent with the suggestion by Fisher (1965) that southern South Island *R. sericophyllus*-like species were ancestors of the locally restricted species *R. pachyrrhizus* (Fig. 2). This species has evolved small, coriaceous leaves and has expanded the distribution of its *R. sericophyllus*-like ancestor eastward to central Otago (Fig. 2), a region that has colder and drier winters than the habitat currently occupied by *R. sericophyllus*. *Ranunculus viridis*, native to Stewart Island (Figs. 1, 2), also appears to have originated locally, again from the southern part of the *R. sericophyllus* distribution (Figs. 10, 11).

Further, the splits graphs identify two distinct geographical groupings of genetically most closely related taxa (Figs. 8–11), one in the southern South Island and one in the central South Island. This observation may suggest the existence of two glacial refugia, from which species such as *R. viridis*, *R. pachyrrhizus*, and *R. grahamii* have evolved.

RANGE EXPANSION OF *RANUNCULUS* SECT. *PSEUDADONIS* IN NEW ZEALAND

The South Island southern alps contain most of New Zealand's endemic alpine plant species (McGlone, 1985; Wardle, 1988). Consistent with this pattern, most species of alpine *Ranunculus* also occur in the South Island. It is there that the greatest genetic diversity between species as well as among populations has been identified in our investigations. On the North Island of New Zealand only *R. insignis*, *R. verticillatus*, and *R. nivicola* occur. J_{SA} sequences from *R. insignis* sampled from geographically distant North Island populations have been found to be identical (unpublished obs.). Similarly, North Island ITS sequences for geographically separated populations of *R. verticillatus* have also been found to be very similar (unpublished obs.). If diversification of the four major lineages occurred in the South Island during the Pliocene (within the last 5 My), then the dispersal of both *R. insignis* (from Fisher's *few petals, coarse hair* breeding group) and *R. verticillatus* (from Fisher's *many petals, silky hair* breeding group) into the North Island might have easily occurred during the Pleistocene when snowlines were depressed and a lowering of the sea level by ca. 120 m connected the sounds region of Marlborough with the west coast of the North Island (Fisher, 1965; Te Punga, 1953; Fleming, 1962). During the Pleistocene the South Island of New Zealand was also connected with Stewart Island in the south. Thus the same dispersal mode as for *R. insignis* and *R. verticillatus* might also be suggested for *R. viridis*, a species for which analyses of J_{SA} and ITS sequences suggest

derivation from southern populations of a species ancestral to *R. sericophyllus* (Figs. 10, 11).

An interesting observation is that the North Island distribution of *R. verticillatus* is restricted relative to that of *R. insignis*. Further, the North Island endemic and allopolyploid *R. nivicola* extends the range of these lineages into higher altitudes, as well as into more western and northern North Island alpine regions (Fig. 2). Previously, Fisher (1965) suggested that *R. nivicola* ($2n = 96$) originated via an allopolyploid event between the northward migrating populations of *R. insignis* ($2n = 48$) and *R. verticillatus* ($2n = 48$). The relative position of *R. nivicola* in our ITS and J_{SA} trees (Figs. 6, 7) confirms this hypothesis.

PARALLEL EVOLUTION OF SIMILAR MORPHOLOGIES

Our molecular phylogenies (Figs. 6, 7) provide evidence for a number of convergent morphologies that support Fisher's (1965) argument that the colonization of similar habitats by species from different breeding groups has occurred with the independent evolution of similar adaptations. These include (a) the reflexing of pedicels downward between the leaf blades during ripening of the fruits, a possible adaptation to loose sandy soils, in *R. crithmifolius*, *R. acaulis* (lowland taxon, sect. *Epirotes*), and also in *R. recens* (sect. *Chrysanthe*); (b) stiff petals in species occupying windswept habitats, for example in *R. pinguis* (Campbell and Auckland Islands) and in the distantly related *R. subscaposus* Hook. f. (sect. *Chrysanthe*) of the Auckland Islands (Fisher, 1965); (c) the development of large entire fleshy leaves, as a possible adaptation to low irradiation and high humidity habitats, in *R. pinguis*, *R. lyallii*, and *R. insignis*; and (d) the presence of deep rhizomes, as a possible adaptation to loose screes, in *R. crithmifolius*, *R. scrithalis*, and *R. haastii*.

Convergence in morphology between species even from different genera is a notable feature within the family Ranunculaceae. For example, in the context of the phenotypic states described above, similar structures are recorded from *Anemone blanda* (reflexed pedicels), *Anemonopsis macrophylla* (stiff petals), and *Caltha palustris* (large fleshy leaves) (Ulbrich, 1905). However, note that in other cases, within the alpine Ranunculi of New Zealand, evolution of morphological characters is not convergent and a number of morphological characters are congruent with our molecular phylogeny. These include achene shape, which distinguishes section *Pseudadonis* from section *Chrysanthe*. *Few petals* (5–8) and coarse hairs on leaves, scapes, and achenes are characteristic of species in group III (Figs. 6, 7). *Many petals* (7–16) and silky hairs on leaves, scapes, and achenes characterize species in groups I and II (Figs. 6, 7). However, note the exception of the few-petalled *R. sericophyllus* in this group, which is furnished with three distinct nectary glands per petal suggesting the hypothesis of former fusion of smaller petal primordia (Fisher, 1965). The apparently paraphyletic *R. lyallii* also shares white flowers with its close relatives *R. anemoneus* and *R. buchananii*, but these species (all group I species) do not form a monophyletic clade in our phylogenetic graphs.

SUMMARY

The alpine Ranunculi of New Zealand are a well circumscribed plant group having undergone diversification and range expansion during a period of dramatic geological and climatic change. The molecular analyses we report here confirm many of the hypotheses of Fisher (1965) as well as suggesting some new hypotheses. The observations of reticulation in our ITS splits graphs for diploid species and our confirmation of the allopolyploid nature of *R. nivicola* raises the question whether interspecies hybridization may have been causal in range expansion in a number of instances (Ehrendorfer, 1958; Stebbins, 1984). The origin of the group remains unclear, and this question, as well as the suggested patterns of diversification, needs to be tested with additional intraspecies sampling and higher resolution methods that might better assess the extent of genome introgression (e.g., Wolfe et al., 1998). The approach we have used to find a highly variable molecular marker in the alpine Ranunculi of New Zealand appears useful for obtaining PCR markers for studying other plant groups (e.g., McLenachan et al., 2000). Split decomposition, also used here, has potential for investigating other Late Tertiary–Quaternary radiations. The method is expected to be particularly useful when characterizing potentially hybrid taxa and/or when investigating recently diverged groups. In the present study, we omitted heteroplasmic sites before calculating our phylogenetic graphs, and this general approach is expected to result in loss of valuable information that may indicate hybrid complexity in some taxa. As recently discussed (Huber et al., 2001), a current research interest includes examining how these sites might be incorporated into analyses using both split decomposition and median graphs.

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OVERVIEW OF THE NEOTROPICAL GENUS *NOTOPLEURA* (RUBIACEAE: PSYCHOTRIEAE), WITH THE DESCRIPTION OF SOME NEW SPECIES[1]

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Abstract

Notopleura (Benth. & Hook. f.) Bremek. is a neotropical genus of about 73 species of herbs and subshrubs, both terrestrial and epiphytic, found in wet neotropical forests from Mexico and the Antilles to Bolivia and Brazil. This genus was formerly included in *Psychotria* L., from which it differs in its low succulent habit, distinctive stipule morphology, usually pseudoaxillary inflorescences, and pyrenes with two germination slits on the adaxial face. Presented here are a description of the morphology of *Notopleura*, a key and enumeration of its species, nomenclature including synonymy and new combinations for 2 subgenera, 53 previously described species, and 4 infraspecific taxa, and descriptions and illlustrations of 18 new species.

Key words: neotropical flora, *Notopleura*, *Psychotria*, Psychotrieae, Rubiaceae.

Notopleura (Benth. & Hook. f.) Bremek. comprises about 73 neotropical species of terrestrial and epiphytic herbs and subshrubs from central Mexico and the Antilles to Brazil and Bolivia. This group was first recognized taxonomically as *Psychotria* sect. *Notopleura* Benth. & Hook. f. in 1874. Bremekamp (1934) first treated this group as a separate genus; subsequently, Steyermark (1972) returned to including this group as a section of *Psychotria* subg. *Heteropsychotria* Steyerm. More recent studies agree that *Notopleura* is after all better treated as a genus separate from *Psychotria* based on both morphological (Taylor, 1996) and molecular (Nepokroeff et al., 1999; Andersson & Rova, 1999) evidence. *Notopleura* is distinguished from *Psychotria* by its low, succulent, often unbranched or clambering habit; its stipules united around the stem into a reduced to well-developed sheath with a single, often glandular, interpetiolar appendage of varied form that is inserted below the top of the sheath; its inflorescences that are pseudoaxillary in most species; and its pyrenes with two small germination slits on the basal end of the adaxial (ventral) face. *Psychotria* does include species with varied habits including a few herbs, species with stipules that are sometimes united around the stem into a continuous sheath, and species with sometimes pseudoaxillary inflorescences. However, stipules with a medial appendage are unique to *Notopleura*.

Here I review the morphology of *Notopleura*; provide a list (see Appendix 1) of its component species and infrageneric classification and keys to both of these; make the necessary nomenclatural combinations to separate *Notopleura* from *Psychotria*; and describe several new species. *Notopleura* has not been treated in its entirety since Bremekamp (1934) included 4 species. Here 2 new subgenera are recognized and 69 species are added. In general, members of subgenus *Notopleura* are terrestrial and have pseudoaxillary inflorescences, while those of subgenus *Viscagoga* are epiphytes with inflorescences that may be terminal or pseudoaxillary. About a fourth of the *Notopleura* species are newly described in this article; these are variously distributed from Guatemala through Bolivia.

Morphology of *Notopleura*

Plants of *Notopleura* are rather succulent, usually unbranched or little-branched herbs or subshrubs 1 m tall or shorter, though a few species may reach 3 m tall. The terrestrial species (subg. *Notopleura*) are found in the understory of wet forest, most commonly in wet microsites such as swamps and stream edges, while the epiphytic spe-

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